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GENETIC VARIATION IN BLUE WHALES IN THE EASTERN PACIFIC: IMPLICATION FOR TAXONOMY AND USE OF COMMON WINTERING GROUNDS

LeDuc, R.G.[1*], Archer, E.I.[1], Lang, A.R.[1], Martien, K.K.[1], Hancock-Hanser, B.[1], Torres-Florez, J.P.[2,3], Hucke-Gaete, R.[3,4], Rosenbaum, H.R.[5,6], van Waerebeek, K.[7], Brownell, Jr., R.L.[1], Taylor, B.L.[1]

1. Marine Mammal and Turtle Division, Southwest Fisheries Science Center, National Marine Fisheries Service, NOAA, 8901 La Jolla Shores Drive, La Jolla, CA, USA
2. Departamento de Genética e Evolução, Universidade Federal de São Carlos, Rod. Washington Luis, km 235, São Carlos, SP, Brazil
3. Centro Ballena Azul / Blue Whale Center, Valdivia, Chile
4. Instituto de Ciencias Marinas y Limnológicas, Universidad Austral de Chile, Casilla 567, Valdivia, Chile
5. Ocean Giants Program, Wildlife Conservation Society – Global Conservation, 2300 Southern Blvd. Bronx, NY 10460, USA
6. Sackler Institute for Comparative Genomics, American Museum of Natural History, NY, USA

7. Peruvian Centre for Cetacean Research, Lima 20, Peru

* Corresponding author: Rick LeDuc, SWFSC 8901 La Jolla Shores Dr. La Jolla, CA 92037, 858-546-7003, casaleduc@gmail.com

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ABSTRACT

Many aspects of blue whale biology are poorly understood. Some of the gaps in our knowledge, such as those regarding their basic taxonomy and seasonal movements, directly affect our ability to monitor and manage blue whale populations. As a step towards filling in some of these gaps, microsatellite and mtDNA sequence analyses were conducted on blue whale samples from the Southern Hemisphere, the eastern tropical Pacific (ETP), and the northeast Pacific. The results indicate that the ETP is differentially used by blue whales from the northern and southern eastern Pacific, with the former showing stronger affinity to the region off Central America known as the Costa Rican Dome, and the latter favoring the waters of Peru and Ecuador. Although the pattern of genetic variation throughout the Southern Hemisphere is compatible with the recently proposed subspecies status of Chilean blue whales, some discrepancies remain between catch lengths and lengths from aerial photography, and not all blue whales in Chilean waters can be assumed to be of this type. Also, the range of the proposed Chilean subspecies, which extends to the Galapagos region of the ETP, at least seasonally, perhaps should include the Costa Rican Dome and the eastern North Pacific as well.

INTRODUCTION

There are many unresolved questions surrounding the taxonomy of blue whales (*Balaenoptera musculus*). During the years of commercial whaling, especially in the Southern Hemisphere, geographical variation was noted (Mackintosh & Wheeler 1929), but not until Ichihara (1966) was one of the variants formally described as a subspecies. In that paper, data from whales hunted from the southern Indian Ocean were used to describe the "pygmy" subspecies (*B. m. brevicauda*), which was distinguished from the Antarctic, or "true" blue whale (*B. m. intermedia*) by its shorter length and different body proportions (essentially, shorter overall and with a shorter tail region). This distinction between the two subspecies has since been widely accepted. However, what is often forgotten is that the relationship between the two subspecies is still incompletely understood. If we follow the concept as used by Branch *et al*. (2007a), a subspecies is "a group of individuals from the same species that is geographically distinct and recognizably different (in terms of morphology, genetics, or behavior) but could interbreed with another subspecies if they shared the same habitat." Implicit in this definition is some degree of geographic separation of gene pools – reduced interbreeding allows the recognizable differences to develop. Unfortunately, the vast majority of blue whale data and specimens, including those analyzed by Ichihara (1966), were collected during the non-breeding season (i.e., summer). During this season, there is a general segregation of subspecies, with the Antarctic subspecies being south of the Antarctic convergence around the Antarctic continent, and the pygmy subspecies described by Ichihara (1966) feeding primarily north of this convergence in the southern reaches of the Indian Ocean (Kato *et al*. 1995). However, this summer segregation has no direct relevance to the subspecies status of the two forms. Each subspecies was thought to migrate to lower latitudes during the austral winter, but knowledge of specific breeding ranges, critical to understanding subspecies relationships, is nearly absent. While this gap in our knowledge does not necessarily challenge the subspecies status of *B. m. brevicauda* and *B. m. intermedia*, not understanding how and to what degree the gene pools are segregated does mean that our understanding of blue whale subspecies is incomplete. As summarized by Rice (1998), there is uncertainty of the subspecies identity of blue whales from the northern Indian Ocean and the southeast Pacific Ocean. A subspecies name (*B. m. indica*) had been proposed for the northern Indian Ocean type; however, other than a greater body length for two specimens (it is rare in collections), little was known to distinguish it from other subspecies. Branch *et al*. (2007a) considered these whales to likely belong to the subspecies *B. m. brevicauda*. The blue whales from the Humboldt Current off western South America, geographically disjunct but similar in body proportions to Indian Ocean pygmy blue whales, were tentatively included by Rice (1998) in the subspecies *B. m. brevicauda*. It was unknown if there was any exchange between the nominal pygmy blue whales, who spent summers in the southern Indian Ocean, and these other populations. In other words, is the pygmy subspecies this geographical variant limited to the southern Indian Ocean (in summer) or does it have a much broader range? The lack of data precludes any strong conclusions. This gap in our knowledge could conceivably have larger taxonomic implications. In a hypothetical example, if the pygmy and Antarctic blue whales were found to be sympatric during the breeding season, one could make the case that the morphological differences were likely maintained by reproductive barriers, and they would qualify as separate biological species.

In a previous paper (LeDuc *et al*. 2007), genetic data were used to understand large scale (i.e., ocean basin) variation among blue whales in the Southern Hemisphere and how it applies to relationships among subspecies. The conclusions drawn from that paper were limited for a variety of reasons, not the least of which is the lack of a reliable method for dividing the sample set by subspecies. Historically, there was concern that there was some degree of mixing during the summer, especially in Antarctic waters, where some of the whales taken during commercial whaling were thought to be of the pygmy subspecies. It was thought that some pygmy blue whales could wander far to the south during the summer months and end up in Antarctic waters. However, Branch *et al*. (2007a) and Branch et al. (2009) estimated rates of mixing from catch data to be less than 1%, perhaps not even differing significantly from zero. Without comparable evidence from body measurements, modern estimates of mixing (Kato *et al*. 2002; LeDuc *et al*. 2007) are even more uncertain. With a sample set largely based on biopsy samples from live whales taken during the summer feeding season, the data in LeDuc *et al.* (2007) lacked both a ground truth of body morphometrics and information about breeding range of individuals. In other words, the subspecies identity of any given sample, and hence its membership in a particular gene pool, could not be determined conclusively *a priori*. Feeding ground locality was therefore used as a proxy for subspecies/population membership, and population structure within ocean basins was not addressed. In spite of these problems, the data from that study showed a notable degree of genetic divergence among sample strata from different ocean basins, with the Antarctic, Indian Ocean, and southeast Pacific groups all being significantly different from each other to a similar degree. Mixing during the summer season was probably occurring at quite low levels, if it had occurred at all.

In recent years, there has been a challenge to the taxonomic status quo. Based on body measurements taken from commercial whaling catches, Branch *et al*. (2007a) resurrected an idea proposed by Clarke *et al*. (1978); that the Chilean blue whales represent a subspecies separate from *B. m. intermedia* and *B. m. brevicauda*. Their data, derived from sexually mature females, showed the Chilean blue whales to be intermediate in length between the pygmy form and the Antarctic form. Subspecies recognition of these SE Pacific blue whales is compatible with the overall pattern of genetic differentiation in the Southern Hemisphere (LeDuc *et al*. 2007) and with acoustic differences seen in recording of calls from the different areas (McDonald *et al*. 2006). Branch *et al*. (2007a) also raise the possibility that blue whales from the eastern North Pacific may be more closely related to the Chilean form than they are to pygmy blue whales, even though their body proportions are similar to the Indian Ocean subspecies. An important step in unlocking the relationship of the Chilean blue whales to those of the eastern North Pacific therefore is to examine the blue whales inhabiting the eastern tropical Pacific (ETP), the likely wintering grounds for whales from both hemispheres. Blue whales inhabit the ETP, a highly productive region spanning over 28 million km^2 from Central to South America, year-round (Reilly & Thayer 1990; Palacios 1997), primarily in two separate areas: the Costa Rican Dome (an area off Central America with a strong and shallow thermocline (Fielder 2002)) and the waters off Peru and Ecuador (Galapagos Archipelago). In this paper, we present a comparison of blue whale samples from different parts of the eastern Pacific, and provide a context of their relationship within the pattern of variation in the Southern Hemisphere.

MATERIALS AND METHODS

All the data used in LeDuc *et al*. (2007) were included, as well as more recent skin samples from Antarctica, Australia and the eastern Pacific. Data on Chilean whales that were not used in LeDuc *et al*. (2007) were from Torres-Florez *et al*. (2014a). As before, the data were stratified into large-scale geographic areas: Antarctica (ANT), Indian Ocean (IO), eastern South Pacific (ESP), eastern North Pacific (ENP), and ETP. Sample sizes are given in Tables 1 and 2. The ETP is the only known wintering ground among the five strata, and most of the samples from there were collected in September-November, corresponding to a boreal late summer- fall and an austral late winter-spring. Figure 1 shows the collection locations of the samples used in this study.

Data were generated and analyzed as in LeDuc et al. (2007); each sample was sequenced for a 400bp fragment of the mitochondrial control region, sexed according to Fain and LeMay (1995) and genotyped for seven polymorphic microsatellite loci: ACCC392, GATA028, GATA098, GATA417, GT023, EV37 and DlrFCB17 (Buchanan *et al*. 1996; Valsecchi & Amos 1996; Palsbøll *et al*. 1997; Bérubé *et al*. 2000). Random samples (10%) were genotyped twice as a data check, with 100% matching. As in LeDuc *et al*. (2007), pairwise population comparisons using F_{st} for the mitochondrial haplotype data were performed using Arlequin v. 3.5 (Excoffier *et al*., 2005). For the genotype data, GenePop v. 3.1c (http://wbiomed.curtin.edu.au/genepop/index.html) was used for all population analyses, including testing for hardy-Weinberg equilibrium and examining genic (*i.e*., allele frequency) differentiation between population pairs for each locus separately as well as across all loci using Fisher's exact test (Raymond & Rousset, 1995). In addition, ϕ_{st} analyses of the sequence data were conducted using Arlequin 3.5 (Excoffier *et al*. 2005), using the Tamura-Nei model for genetic distance, which was determined by jModelTest v. 2.1.1 (Darriba *et al*. 2012) to be the most appropriate for this dataset. The median-joining network (Bandelt *et al*. 1999) was generated using PopART v. 1.7 (Population Analysis with Reticulate Trees), available at http://popart.otago.ac.nz.

Sex-biased dispersal was examined by conducting the same F_{st} and ϕ_{st} tests of population subdivision for each sex separately with 1000 permutation replicates to assess statistical significance. Additionally, the significance of the difference between sexes for each test statistic was assessed with a secondary permutation test in which a null distribution was created by randomly reassigning the sex of each individual for 1000 replicates. The p-value for the test was the fraction of replicates where $|X_m – X_m|$ was >= the observed $|X_m – X_f|$ with X being either the estimated F_{st} or ϕ_{st} statistic value.

Assignment tests for ETP samples were conducted using STRUCTURE v. 2.3 (Pritchard *et al*. 2000). In this analysis, prior population information was incorporated only for samples from the ENP and ESP, and not for ETP samples, and two populations were assumed. In other words, ETP samples had to be assigned to either one of the source populations from higher latitudes. The analysis also assumed no admixing and no correlation of alleles.

RESULTS

Genetic diversity (e.g. heterozygosity) ranged between 0.690 and 0.751 for microsatellite loci, while for mtDNA sequences it ranged between 0.705 and 0.967 and nucleotide diversity ranged from 0.003 and 0.019 (Table 1). As found previously by LeDuc *et al*. (2007), the Antarctic and the Indian Ocean respectively have the highest and lowest levels of genetic diversity among the different strata. Although population reduction from commercial whaling can have an effect on levels of genetic diversity, the patterns of diversity seen here are more likely a result of past climatic changes and population dynamics (Attard *et al.* 2015). The Supplemental Materials include the GenBank accession numbers for all mitochondrial haplotypes identified (SM Table 1) and sex and haplotype data for each sample (SM Table 2).

Using the microsatellite data for all pairwise comparisons of allele frequencies, the five initial strata (ANT, ENP, ESP, ETP, and IO) were significantly different from each other. However, the apparent distinctiveness of the ETP stratum is not straightforward, as it likely does not represent a distinct breeding population. Because it is the only known wintering ground among the five strata, and known to be visited by whales from both the north and south (Calambokidis *et al*. 1990; Reilly & Thayer 1990; Mate *et al*. 1999; Torres-Florez *et al*. 2014b; 2015), the ETP stratum may actually be comprised of elements of both the ENP and ESP. If their respective contributions were similar in degree, that may have been enough to render the ETP significantly different from both.

In the results from the STRUCTURE analysis, there was an apparent difference in the assignments of samples from the Costa Rican Dome from those originating off the coast of Peru and Ecuador, with the former seeming to be more commonly assigning to the ENP and the latter to the ESP. Figure 2 shows the assignment probabilities of ETP samples from the two regions. The northern ETP samples (nETP) had an average assignment of 0.707 to the ENP and 0.293 to the ESP. Conversely, the southern ETP samples (sETP) had an average assignment of 0.323 to the ENP and 0.677 to the ESP. We tested the difference in the distribution of assignment probabilities within the ETP using the log-odds values of the assignment probabilities, doing a 2-tailed t-test using 2-sample unequal variance. The difference in values between nETP and sETP was significant ($p < 1 \times 10^{-5}$); the difference in means of the log-odds values was 2.6634. Therefore, in subsequent population comparisons, the ETP was subdivided into the nETP and the sETP. Table 2 shows the results of comparisons among the different strata, including a subdivided ETP, based on the microsatellite data. Table 3 shows the results of analysis of the sequence data comparing the large-scale geographic regions, and Table 4 shows the results of similar comparisons using just the regions within the eastern Pacific.

Figure 3 presents the median-joining network derived from the sequence data. Both the Antarctic and Indian Ocean strata share few haplotypes with other strata, whereas the ENP, ETP and ESP share a number of haplotypes. The Indian Ocean samples are the least spread out along the network, while the Antarctic stratum contains the greatest number of haplotypes, occupying widely disparate positions in the network. This is consistent with the Antarctic population having the historically largest population size; this has carried into contemporary high levels of diversity, in spite of depletion by commercial whaling.

DISCUSSION

Composition of the ETP

In the STRUCTURE results, there was a significant tendency for the samples from the southern part of the ETP (the waters of Peru and Ecuador) to assign to the ESP, and the samples from the northern part of the ETP (the Costa Rican Dome and the waters of southern Mexico) to assign to the ENP (Figure 2). This is consistent with the results reported previously for the ESP by Torres-Florez *et al*. (2014b), and with the finding presented in Torres-Florez *et al*. (2015) of an individual whale being sampled both in Chilean waters and in the southern ETP. In spite of this statistical difference, the pattern of individual assignments was ambiguous, with some individuals having strong assignments to the “source” population from the opposite hemisphere, and others having equivocal assignments probabilities (i.e., closer to parity). Even with our assumption of only two source populations, this ambiguity is not surprising, as the two source populations, while significantly different, are not dramatically so (p=0.049 using genic differentiation). There are many shared alleles between the ENP and ESP, and the level of differentiation between them is much less than the levels seen in comparisons involving the Antarctic or Indian Ocean samples. Put simply, there is a statistically significant difference in the assignment of ETP samples, with those from the Costa Rican Dome tending to be assigned to the ENP and those from the waters of Ecuador and Peru tending to be assigned to the ESP. This is not to say that all whales frequenting the sETP come from the ESP and all those in the nETP come from the ENP. Some trans-equatorial movement between the ETP areas is certainly plausible and has been recently documented (Annie Douglas, Cascadia Research Collective, pers. comm.); though the gap between the sETP and nETP is over 400 nmiles, this is not likely to represent much of a barrier to whales that have already travelled thousands of miles. However, it does reflect an overall pattern of use, at least during the season encompassed by the samples, with the sETP being primarily visited by whales moving up from Chilean waters or other parts of the ESP, and the nETP being primarily used by whales from the ENP.

As seen in Table 2, this pattern (affinity between ESP and sETP, and between ENP and nETP) was also apparent when the population analyses comparing overall allele frequencies were repeated with the nETP and sETP being treated as separate strata. The nETP was not significantly different from the ENP, but was different from both the sETP and the ESP. Similarly, the sETP was not significantly different from the ESP, but was from the ENP. These results seem to support the aforementioned pattern of use within the ETP. If the different regions of the ETP were being utilized by whales from different populations, one might expect the mixed stratum of the entire ETP to reveal itself in Hardy-Weinberg disequilibrium of microsatellite alleles. However, tests for Hardy-Weinberg disequilibrium were inconclusive. The ETP *in toto* had only one locus that was significantly out of equilibrium, no more than other strata (each had one locus, save for the ESP, which had zero). This result could reflect a lack of power of the test itself and/or it could stem from the fact that the two component populations of the ETP themselves are not very different from each other.

All comparisons of the control region sequence data among major regions outside the ETP were, as expected, significant (Table 3). In comparisons using the ETP in its entirety, this stratum was usually significantly different from both the ENP and the ESP, only the genetic distance comparison with the ENP was not. This apparent distinctness is plausible if the entire ETP is considered to be an amalgam of portions of two populations – the ESP and ENP; the ETP then may contain enough of each of the source populations to come out as different from both. However, when the analysis included a subdivided ETP being compared to populations to the north and south (Table 4) the results were less congruent with the microsatellite results. For example, the significant difference between the ENP and the nETP in haplotype frequencies is not consistent with the microsatellite results, nor is the significant difference between the ESP and sETP using genetic distances. These differences suggest that the links between ENP and nETP and between ESP and sETP may not be as strong as the microsatellite analysis indicated. In addition, the non-significance of the sequence comparisons between the nETP and sETP suggest that the segregation of those two areas may not be as great as the microsatellite data indicate. However, findings of non-significance involving these areas must be considered in the context of reduced analytical power due to the smaller sample sizes resulting from subdividing the ETP stratum.

The maternal inheritance of the control region sequence data raises the possibility that these results may stem from differences between the sexes in the use of the ETP. In one scenario, not all females from either the ENP or the ESP would venture to the ETP, some instead utilizing other wintering grounds, such as the Gulf of California for northern whales (Sears *et al*. 2013) or an as-yet unknown area. Site fidelity has been seen for female blue whales frequenting the Gulf of California (Sears *et al*. 2013). If this pattern of differential seasonal movement was passed down along maternal lines, the ETP sample of females could contain a non-random sample of the mitochondrial population sampled at higher latitudes. A converse scenario is also possible, whereby the ETP is also visited by females from an area at higher latitudes that is not adequately represented in the dataset. The present data do not address these possibilities, or the possibility of a resident population within the ETP. The one individual sampled at both high (Chile) and low

(Ecuador) latitudes was a female (Torres-Florez *et al.* 2015); although anecdotal, this supports the statistical results that indicate that there is at least some connection between these regions.

Another aspect of blue whale migration is the temporal component. Although effort is not uniform throughout the year, re-sightings of individual blue whales in the Costa Rican Dome (nETP) that were formerly sighted in the ENP occurred during the months of January to March, the boreal winter (Chandler & Calambokidis 2004). These same months correspond to the austral summer and early fall, when blue whales occur off the coast of Chile (Hucke-Gaete *et al*. 2004, Torres-Florez 2011). With one exception (a January stranding from Peru), all the ETP samples in the present study, north and south, were collected during the months of September to November. Whales in low latitudes during this period could represent early winter visitors from the ENP, or late winter visitors from the ESP. Sampling during other months may reveal a different pattern or some complications to the present results. Van Waerebeek *et al*. (1997) documented some of what is known from blue whales in Peruvian waters. We did not find a pattern of variation in assignment probabilities within this three-month time period. The Peruvian sample collected in January had an assignment probability of 0.604 to the ENP, which is consistent with the use of the ETP by whales of the ENP during the boreal winter. However, this assignment probability is too equivocal to conclude that the stranded whale was actually a Northern Hemisphere animal. Any temporal variation in the use of wintering grounds, or use by other populations as yet unsampled, will need to be addressed by future research.

The conventional narrative about blue whale movements reflects the generalized pattern for rorquals, that there are seasonal migrations between high latitude summer feeding grounds and low latitude wintering grounds. However, unlike many other whale species, blue whales migration patterns can change over time (Calambokidis *et al*. 2009). This pattern of seasonal latitudinal movement is thought to apply to both the Antarctic subspecies (*B. m. intermedia*) and the pygmy subspecies (*B. m. brevicauda*) from the Indian Ocean, with requisite differences in their respective ranges. Branch *et.al.* (2007b) summarized much of the historical research on southern blue whales, including sightings, catches, discovery tags, and acoustic recordings. Although much of the data, such as seasonal shifts in abundance across latitudes, supported this narrative, the species overall defied easy characterization. For example, one exception to this pattern was the proposed subspecies of the northern Indian Ocean (*B. m. indica*), which is characterized in part by its non-migratory habits. Furthermore, blue whale calls are detected year-round in Antarctic waters (Širovic´ *et al*. 2004), and calls typical of Chilean blue whales were detected in all months in the ETP (Buchan *et al.* 2014a). These findings suggest that some individuals either do not undertake the seasonal migrations or that they make the journey multiple times within a year.

Considering the results of the genetic analyses presented here, along with the acoustic evidence of ESP whales being present in all seasons (Buchan *et al*. 2014a), it seems likely that the ETP is occupied, at least seasonally and perhaps year-round, by blue whales from both the Northern and Southern Hemispheres. Their relative abundances would change with the seasons, each population becoming more numerous during its respective winter, and would also vary geographically between the nETP and the sETP. If this pattern of overlap is the case, its effect on estimates of abundance in the ETP has important implications for the assessment and management of blue whale stocks.

Often implied in the interpretation of seasonal movements is that the wintering grounds serve as an area for breeding, calving, and/or nursing. This may be true in part – small calves have been recorded in the ETP (for example, Pitman *et al.* 2007) – but data on the key behavior of breeding is lacking (Sears *et al*. 2013). If the ETP does serve as a breeding area, it is possible that offset seasonality in the reproductive status of whales from different hemispheres could explain how the northern and southern populations maintain their distinctness – northern and southern whales could co-occur in the ETP but would be unlikely to interbreed. Indeed, any whale that dispersed to the opposite hemisphere would face the problem of being reproductively out of sync with its adoptive population. Year-round visitation of the ETP also implies that at any given time not only are some of the ETP visitors not in breeding condition, but that some whales migrate to lower latitudes for non-breeding purposes. Blue whales do tend to winter in areas of high productivity (Reilly & Thayer 1990; Branch *et al.* 2007b), and wintering areas serve in part as important feeding areas, and not just for females with calves (Sears *et al*. 2013). But do they represent a place to find mates? This is undetermined.

A clue to the seasonal component may perhaps be seen in Tables 2 and 4, which indicate those comparisons that were significantly different when the sexes were analyzed separately. Initially, it appears that the sex-specific differences are a mosaic with little evidence of a pattern, especially for males. However, in every comparison of females from different strata that was significant, one of the strata was the ENP. Furthermore, the only significant differences found involving the ESP females were those comparing them to the ENP. This suggests that at the time of year of sampling (boreal fall and austral spring), most of the females present in the ETP were from the ESP and not from the ENP. This in turn would suggest that the southern females are the last to leave their wintering grounds and northern females are not among the early arrivals. It is possible that the demands of reproduction impel females to be more regular in the seasonality of the movements – males from either hemisphere would be more likely to visit the ETP during non-winter months. According to this scenario, the ETP during the months of September to November would include a varying mixture of males from both the ENP and the ESP, as well as females from primarily the ESP. Each group would favor the part of the ETP closest to their source population (nETP for ENP and sETP for ESP), but may also "spill over" to the other part of the ETP. This would also be consistent with our sETP stratum having the largest skew in sex ratio, where 2/3 of the samples came from females. If females from the ESP are more likely to be in the ETP than those from the ENP, and the sETP is their favored (but not exclusive) wintering grounds, the sex bias fits. All this is highly speculative, especially in light of the moderate differentiation between the source populations coupled with the meager sample sizes; the analytical power to find differences is very limited when some of the sex-specific strata include fewer than ten samples. The actual pattern of affinities and differences may be much more complex. However, it does allow us to predict that if northern whales have similar tendencies, samples taken from the boreal spring/austral fall would show an inverse pattern of differences.

Resolving the relationship between ENP and ESP blue whale populations will require a better understanding of a number of factors, including the possibility of a resident ETP population, whether or not wintering whales from unknown populations are being sampled in the ETP, if there are other wintering areas not sampled, differential female use patterns, seasonality of mating cycles, location of mating, parts of populations that are not migrating, movements in and out of wintering area within a season, and the relative sizes of source populations.

Taxonomy

In examining length data from historical catches, Branch *et al*. (2007a) concluded that blue whales delivered to Chilean shore stations were intermediate in size between the pygmy and Antarctic subspecies. Based on this finding, they proposed the recognition of a new subspecies of blue whale. In the present study, this conclusion is not at odds with the degree of differentiation between Chilean (*i.e*., ESP) blue whales and those of other regions in the Southern Hemisphere. In comparisons using nuclear data, samples from the ESP were differentiated to a similar degree from Antarctic and Indian Ocean samples as those two were from each other. In addition, the sampling of one individual whale in both Chilean waters and in the southern section of the ETP demonstrates that at least some of these whales range into tropical waters, consistent with the sETP being recognized as part of the subspecies range (Branch *et al*. 2007b). This is corroborated by the finding that the sETP and Chile do not show significant differences in microsatellites. However, the picture becomes more complex when the relationship of Chilean whales to those from further north is considered. In the analyses of sequence data, some of the comparisons of the ESP with strata from the ETP or ENP were not significantly different (Table 4). Even a coarse measure such as number of shared haplotypes suggests the same. The Antarctic and Indian Ocean strata share no more than two haplotypes with any other stratum, while the ESP stratum shares as many as eight with strata to the north (ETP). This is shown graphically in Figure 3.

A comparison of ϕ_{st} values (Table 3) also illustrates the modest level of divergence of the ESP from the ENP. In pairwise comparisons of the Indian Ocean stratum (presumably largely comprised of pygmy blue whales) to those from other regions, the average value of ϕ_{st} is 0.365 (range: 0.335-0.394). The comparable average value in comparisons involving the Antarctic stratum (presumably comprised largely of Antarctic blue whales) is 0.189 (range: 0.127-0.335). For comparisons involving the ESP stratum, the average value of ϕ_{st} is 0.166. This is of similar magnitude to that found for comparisons involving the Antarctic. However, this does not mean that the ESP whales are equally differentiated from those of other regions. The ϕ_{st} value for comparisons between the ESP and the Indian Ocean and Antarctica are 0.394 and 0.158, respectively. However, the ϕ_{st} values for the ESP to the ETP and to the ENP are an order of magnitude lower, 0.040 and 0.073 respectively. So while there is still much uncertainty regarding the population structure and movements within the eastern Pacific (discussed above), the present genetic data indicate that at least some Chilean blue whales range into tropical waters and that their degree of differentiation from whales to the north (including the ENP) is considerably less than to other recognized subspecies. At the very least, the current results support Branch *et al*.'s (2007b) suggestion that the range of the proposed Chilean subspecies should include, at least seasonally, the waters of Peru and Ecuador. Furthermore, if the degree of genetic differentiation is to be used as a guide for subspecies inclusion, it should perhaps be extended to include the mid-latitudes of the eastern North Pacific.

There is additional uncertainty involving the Chilean subspecies. In analyses of length data from historical records and from contemporary aerial images, Gilpatrick and Perryman (2008) noted that blue whales from California waters and from both northern and southern sections of the ETP had body lengths nearly identical to those of pygmy blue whales from the Indian Ocean. Recent research by Durban *et al*. (2016) extends that similarity to whales photographed in Chilean waters. This is not at odds with the genetic findings - documented movement between ESP and sETP (Torres-Florez *et al*. 2015) and the modest differentiation between ESP and ENP found in the present study. However, it is at odds with the findings of Branch *et al*. (2007b) that Chilean whales are significantly longer than those of the pygmy subspecies.

There are a few possible explanations for this discrepancy. First, there is a large temporal gap among the studies. Branch *et al*. (2007a) used historical data from whales collected mostly from the 1960s, while Gilpatrick and Perryman (2008) used more contemporary data collected between 1994 and 2003, samples for the present study were collected between 1991 and 2008 (save for a single sample from 1982), and the data from Durban *et al*. (2016) was from 2015. That is not to say that the Chilean blue whales have gotten shorter in the interim; there is however the possibility that localized extirpation did occur, and the range was later occupied by another population of blue whales expanding their range from elsewhere. If this scenario were true, this would mean that the subspecies suggested by Branch *et al*. (2007a) may no longer exist. However, the recorded catches were likely too small to result in extirpation (Williams *et al*. (2011). Of course, this is only one possible explanation.

Aguayo (1974) stated that both pygmy and Antarctic blue whales were landed at Chilean shore stations. The existence of a mixed dataset, which would explain a finding of intermediate body lengths, was tested for and discounted by Branch *et al*. (2007a) for their dataset, but it does illustrate the possibility that multiple forms of blue whales are currently inhabiting and/or transiting Chilean waters. The only locality information in the historical records used by Branch *et al*. (2007a) and Aguayo (1974) refers to the shore station itself and not the actual catch location, so we cannot be sure that the whales sampled for our study (mostly along the coast but some from offshore), were drawn from the same population as those of Branch *et al*. (2007a). In this scenario, the subspecies proposed by Branch *et al*. is still extant, albeit with an unknown range and habitat and not sampled by any of the more contemporary studies. Buchan *et al*. (2014b) reported the existence of two blue whale songs from Chilean waters; however, whether or not this corresponds to separate call types or variation within a call type was not determined. Only additional survey and sampling efforts can address this possibility.

A third possibility is that differences in reported body lengths represent differences in methodology. Catch data and aerial photogrammetry each have their own set of assumptions and biases; whether or not these are sufficient to explain the apparent discrepancy in Chilean blue whale lengths is beyond the scope of this paper. However, it is worth noting that Gilpatrick and Perryman (2008) did not find significant differences between lengths from photogrammetry and lengths from catch data for the ENP, either for the catch as a whole or for just mature females.

Lastly, it may be that current levels of variation simply have not been adequately captured. Gilpatrick and Perryman (2008) had measurements of only two mature females from the sETP, and Durban *et al*.'s (2016) sample size from Chile also included only two mature females. Although the measurements in these studies were similar to each other and to the mean body lengths recorded for the pygmy subspecies, they were within the range of measurements for both Chilean and pygmy whales given in Branch *et al*. (2007b). More length data from contemporary whales and more work on comparing photogrammetry data to data from catches is needed to resolve this issue.

Setting aside the apparent discrepancy in size data, the possibility also exists of Antarctic blue whales transiting up the South American west coast, as indicated by the recording of Antarctic-type calls in low latitudes by Stafford *et al*. (2004) and the report of Aguayo (1974). At the very least, if one recognizes the Chilean subspecies of blue whales as a taxon, it should be kept in mind that not all blue whales occurring in these waters may be of this subspecies. This is an important factor to consider in designing any research program directed at management or population assessment of Chilean blue whales. This caveat also points to the larger issue of delimiting subspecies. Since subspecies are traditionally defined in terms of locally adapted populations or groups of populations, there is an implication of some degree of breeding segregation. However, in the case of blue whales, for which most sampling has occurred outside of the breeding season and for whom breeding ranges are often unknown, this critical part of delimiting subspecies is lacking. For example, the Chilean subspecies may actually breed in the ETP, and may or may not have opportunities for genetic exchange with Northern Hemisphere blue whales that also occur there. If they occur in the same areas but are seasonally offset (either in the timing of their occurrence or of their breeding condition), interbreeding may be rare or negligible and they may represent different subspecies, depending on their degree of differential adaptation. In summary, the breeding range of a subspecies is an integral part of its very definition, and if that range is incompletely known then the delimitation of even valid subspecies is incomplete.

Geographic range is also critical in evaluating potential correlations between morphological and genetic similarities in a taxonomic context. When the pygmy subspecies was proposed by Ichihara (1966), he documented morphological differences from the Antarctic form that were later shown to be congruent with their genetic divergence (LeDuc *et al*. 2007), but the morphological similarities between blue whales from the NE Pacific and the nominal pygmy blue whales from the Indian Ocean were not congruent with their genetic differences presented here. In fact, the closest agreement to date among independent data sets involving comparisons across ocean basins for blue whales is the distinctness of *B. m. brevicauda* exhibited by genetic analysis (LeDuc *et al*. 2007, present study) and by analysis of acoustic data (McDonald *et al*. 2006). At this stage in the study of blue whale taxonomy, it appears that morphological differences have thus far been corroborated by other lines of evidence (e.g., Antarctic blue whales vs. other subspecies), but morphological similarities have not always translated in similarities of other types of data (e.g., Indian Ocean pygmy blue whales vs. ENP blue whales). Of course, how blue whales from the Atlantic Ocean fit into the global pattern of variation will also be a critical part of the resolving the taxonomy. Congruence and incongruence of patterns of variation in comparing different types of data results from the different mechanisms and rates of genetic, behavioral, ecological and morphological divergence, differences that need to be considered when attempting to determine subspecies.

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DATA ACCESSIBILITY:

Data available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.m066r. Data from ESP, sETP and ANT also form part of the data deposited at doi:10.5061/dryad.bc558, which was used in Torres-Florez *et al*. 2014b). The deposit files contain data of each sample with the locality (major area), latitude and longitude, multilocus genotype, sex, haplotype name and Genbank accession number.

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FIGURES AND TABLES

Figure 1. Sampling locations for blue whales used in the present study. ETP samples are shown in the inset.

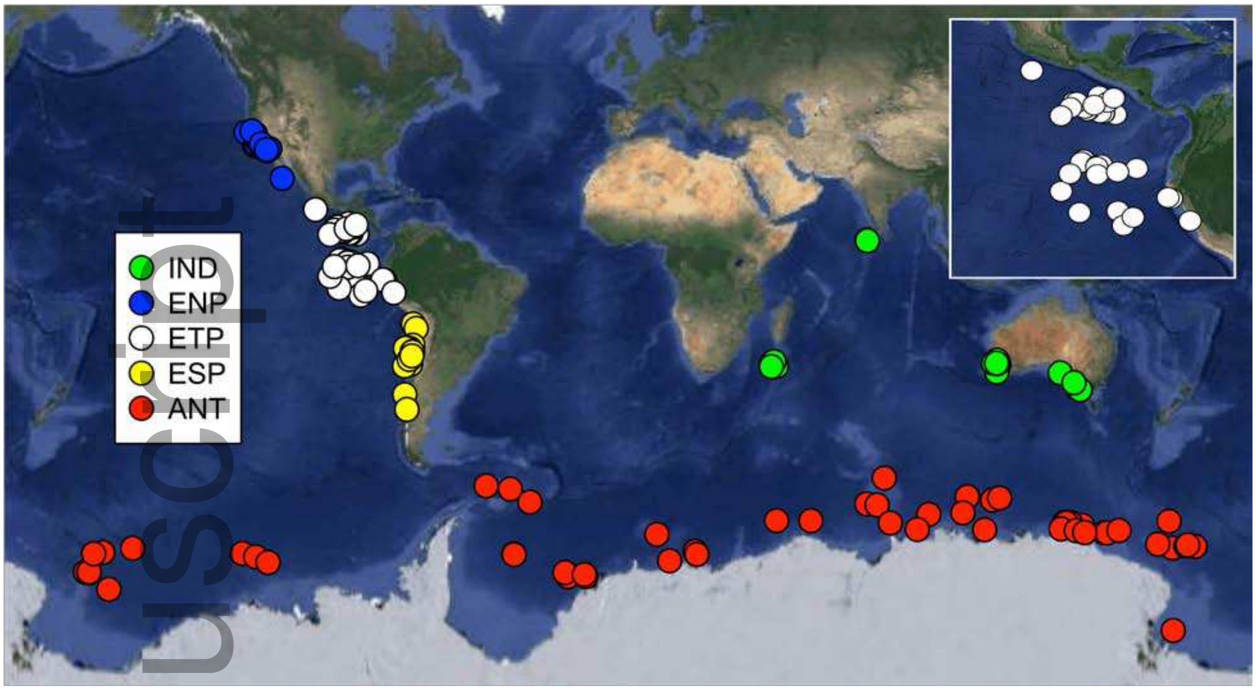
IND
ENP
ETP
ESP
ANT

Figure 2. Fig. x. Relationship between latitude and Pr(assignment to ENP) for samples from the two regions of the ETP (top), and a smoothed density curve of the distribution of the same points along Pr(Assignment to ENP) (bottom).

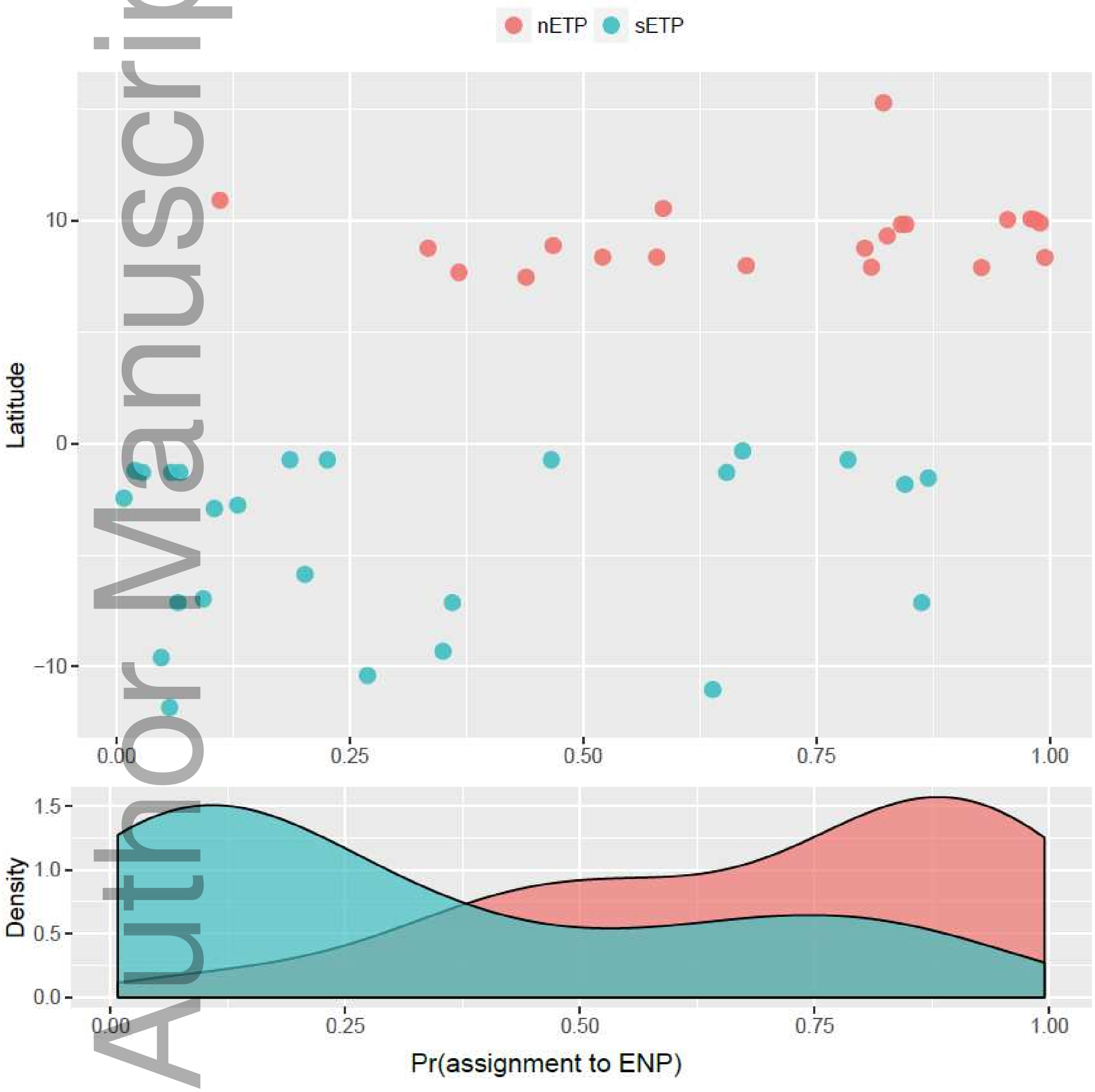

Figure 3. Median-joining network of blue whale haplotypes. Each circle represents a haplotype. Sizes of the circles represent the number of samples from each haplotype. Cross lines represent 1 mutational step.

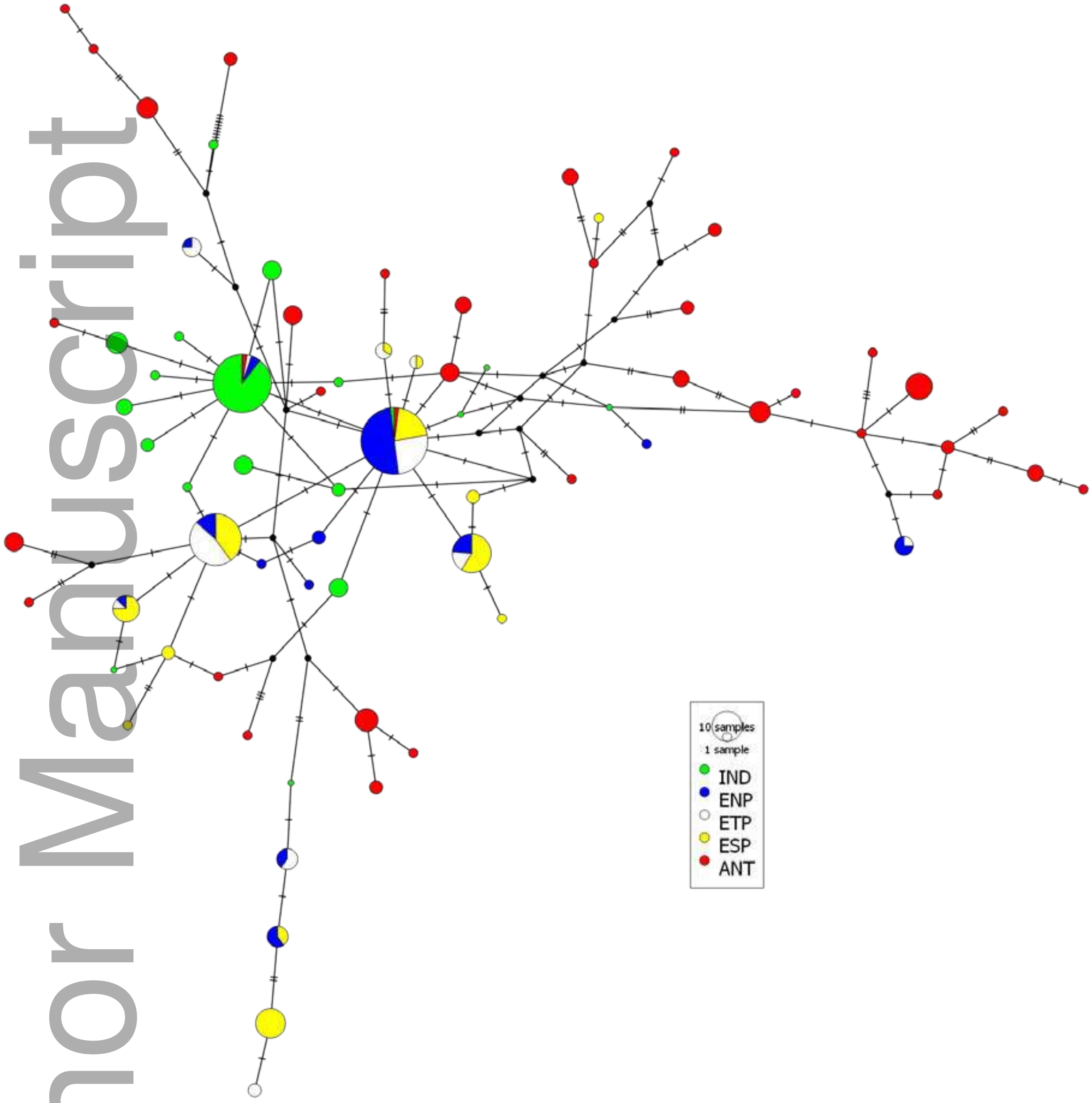
10 samples
1 sample
IND
ENP
ETP
ESP
ANT

Table 1. Summary of the data generated, combining the present data with data from LeDuc *et al*. (2007). N (M, F, U): Number of samples (Male, Female, Unknown), Hobs: Observed Heterozygosity, Vs: Variable/polymorphic sites, H: Number of Haplotypes, Hd: Haplotypic diversity, Pi: Molecular diversity.

	MICROSATELLITES & SEX		**SEQUENCES**				
POPULATION	**N (M/F/U)**	**Hobs**	**N**	**Vs**	**H**	**Hd**	**Pi**
ANT	78 (42/35/1)	0.751	78	41	36	0.967	0.019
ENP	51 (23/28/0)	0.730	50	17	13	0.738	0.007
ESP	66 (23/20/23)	0.723	59	16	13	0.872	0.011
ETP nETP sETP	46 (17/27/2) 21 (9/11/1) 25 (8/16/1)	0.719	44	17	11	0.810	0.007
IO	66 (37/28/1)	0.690	64	13	14	0.705	0.003

Table 2. Population analyses of genotype data: *p*-values from analysis of genic differentiation with samples from both sexes included; *p*-values in bold are those <0.05. An "M" or "F" denotes that the comparison was significant at $p < 0.05$ for the designated sex when the sexes were analyzed separately.

Population	IO	ENP	nETP	sETP	ESP
ENP	**<0.0001 M F**	*			
nETP	**<0.0001 M F**	0.2461 **M**	*		
sETP	**<0.0001 M F**	**0.0001 M F**	**0.0151 M**	*	
ESP	**<0.0001 M F**	**<0.0001 M F**	**<0.0001 M**	0.8192	*
ANT	**<0.0001 M F**	**<0.0001 M F**	**<0.0001 M F**	**<0.0001 M F**	**<0.0001 M F**

Table 3. Analyses of control region sequence data: *p*-values from pairwise population tests; *p*-values in bold are those <0.05. Below the diagonal are the results of ϕ_{st} tests using Tamura-Nei genetic distances (ϕ_{st} values in parentheses). Above the diagonal are the results of F_{St} tests using haplotype frequencies (number of shared haplotypes in parentheses).

Population	IO	ENP	ETP	ESP	ANT
IO	*	**<0.00001** (2)	**<0.00001** (2)	**<0.00001** (1)	**<0.00001** (2)
ENP	**<0.00001** (0.346)	*	**0.00098** (8)	**<0.00001** (5)	**<0.00001** (2)
ETP	**<0.00001** (0.385)	0.28320 (0.003)	*	**0.01074** (6)	**<0.00001** (2)
ESP	**<0.00001** (0.394)	**0.00293** (0.073)	**0.02637** (0.040)	*	**<0.00001** (1)
ANT	**<0.00001** (0.335)	**<0.00001** (0.136)	**<0.00001** (0.127)	**<0.00001** (0.158)	*

Table 4. Analyses of control region sequence data for the eastern Pacific: *p*-values from pairwise population tests. Significant *p*-values are in bold. Below the diagonal are the results of ϕ_{st} tests using Tamura-Nei genetic distances (ϕ_{st} values in parentheses). Above the diagonal are the

results of F_{St} tests using haplotype frequencies. An "M" or "F" denotes if the comparison was significant when the sexes were analyzed separately.

Population (#haplotypes)	**ENP**	**nETP**	**sETP**	**ESP**
ENP (13)	*	**0.02051**	**0.02051 F**	**<0.00001 M F**
nETP (7)	0.10938 (0.030) **F**	*	0.31250	**0.01074**
sETP (7)	0.30762 (0.003)	0.05957 (0.055) **M**	*	0.08887
ESP (13)	**0.00293** (0.073) **F**	0.22266 (0.012)	**0.02637** (0.072)	*